**Comparative study of Pb speciation and behaviour in a accumulator and sensitive plant.**

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## Aims of the experiment and scientific background

Due to its persistence and numerous uses, lead is one of the most common pollutants in the environment (Alkorta *et al.*, 2004) and it can induce negative effects on the health (Henry, 2000; Walker *et al.*, 1996). Some plants present a high Pb sensitivity and could be used as bio indicator of toxicity. On the opposite, others can accumulate large quantities of Pb and could be used in remediation processes (Liu *et al.*, 2005; Panich-Pat *et al.*, 2003). However little is known actually on the involved mechanisms concerning toxicity or resistance of plants to Pb. The speciation of Pb governs its absorption (or adsorption or sequestration), then its translocation (or stoking in the vacuoles for instance) and finally its toxicity. Both soil (physic and chemical parameters) and plant characteristics (species, variety and maturity) influence metal speciation. According to Liu *et al.* (2005 and submitted), a low pH favours the absorption by plants. Producing exudates, the plants can modify the physical, chemical and biological characteristics of its near environment (Paul *et al.*, 1998; Hinsinger, 2001; Bertin *et al.*, 2003) and consecutively the behaviour of metals (Dakora & Phillips, 2002; Salt *et al.*, 2002). The determination of the chemical speciation is therefore a key data in order to progress in the comprehension of the phenomenon (Ferrand *et al.*, submitted).

The purpose of our study is to realise a comparative study of Pb behaviour in rhizosphere, solutions, roots, sap xylem and aerial parts of two different plants: *Vicia Faba* and Attar of Rose *Pelargonium*, respectively high sensitive and resistant plants for lead stress contamination. The studied soil is localized in the vicinity of a lead recycling factory near Bazoches (58, France). The total lead concentration: 1830 mg/kg is in the range considered as "intervention level" in France, but the high pH in relation with the calcareous amount of 2.1% could reduce the mobility of lead. However $Mg(NO_3)_2$ extraction performed on the top soil demonstrated a relatively high availability of the lead (4 mg Pb/kg soil). Culture experiments of various *Pelargonium* cultivars and *Vicia Faba* were realised both in the field and in the laboratory using an experimental device that permit to obtain rhizosphere soil (Guivarch *et al.*, 1999). A *Pelargonium* cultivar (Attar of Rose) that offers high biomass level and translocation rate was selected (1477 mgPb/kg DW). Moreover its crops could be exploited through the production of essential oils. With a $[Pb]_{xylem\ sap}$ of 3.4 mg/L, that cultivar doesn't show any toxicity sign as measured by micro-nucleus or oxidative stress tests. But as the lead concentration increases a hardening of its root tissues was observed, suggesting perhaps a replacing of the $Ca^{2+}$ ions by $Pb^{2+}$ ions (like in the human bones). Moreover, Morard *et al.* (2000) and Baboulène *et al.* (submitted) observed the remobilisation of calcium from the apex zone for stressed tomatoes. At the reverse side *Vicia Faba* strongly reacts during bio toxicity tests, moreover with a $[Pb]_{xylem\ sap}$ of 3.2 mg/L, dark colour grains where observed when the xylem sap was air exposed. We propose to study by EXAFS the speciation of Pb in the roots, xylem sap and aerial parts samples of *Vicia Faba* and Attar of Rose *Pelargonium.* The samples will be prepared taking care of minimize the risk of speciation modification. For instance the freeze-drying will be avoided and the xylem sap samples will be extracted under inert gas (Ar) and stored in a sealed quartz capillary (inner diameter = 3 mm, with minimum death volume) in frozen state. Xylem sap samples will be also concentrated after deposit on a quartz sheet and evaporation and analysed with SEM-EDS technique at the ENSIACET (Toulouse). Various specific zones of leaves and roots samples will be separated and then frozen. Due to the low Pb levels in some samples studied, detection and quantitative analysis of lead species will only be achievable by EXAFS spectroscopy.

## Experimental method

We plan to record EXAFS data at the Pb LIII-edge (13.055 keV) on plant samples with Pb concentrations ranging from few ppm (xylem sap) to 1500 ppm (roots). Recording high-quality EXAFS data on such diluted samples requires a high-flux and a high counting rate fluorescence

detector. 10 plant samples will be prepared: 2 plants (*Pelargonium* and *Vicia Faba*) × [2 root samples (apex and higher zone), 2 leave samples (edge and centre), and xylem sap]. Moreover we need to analyse a set of reference lead compounds (phosphates, carbonates, oxalate, citrate, etc.) prepared in sap matrix (with a concentration near saturation) in order to interpret our results. All the samples will be recorded as frozen hydrated, using a helium cryostat. A long acquisition time is required for the dilute samples like xylem sap (around 8h), around 4h for the roots and around 2h for the reference compounds. That explain why we ask for 15 shifts to realize this project. As plant components are often poorly ordered materials, collecting EXAFS data below 30 K will help in determining the second-neighbor environment which is a key indicator for our purpose. We estimate that 5 days (= 15 shifts) are necessary to realize this experiment.

**Results expected**

Our proposed work is aimed at using EXAFS to in-situ characterize lead speciation in various plant compartments as a function of plant species. Combining EXAFS results with complementary analyses by Micro-SXRF and non-synchrotron-based techniques (XRD, SEM-EDS, toxicity tests and chemical extractions) will provide to determine the mechanisms of lead transport and stocking in the plants. The primary benefit of our work will be improvement of the scientific basis for risk assessment and remediation design for this toxic element. Moreover only few EXAFS data concern the lead in plant material, therefore it seems an interesting aim to increase the reference compounds spectra in that area.

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